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(21) International Application Number: PCT/US92/02440 (22) International Filing Date: 1 April 1992 (01.04.92) (30) Priority data: 678,129 1 April 1991 (01.04.91) US (60) Parent Application or Grant (63) Related by Continuation US 678,129 (CIP) Filed on 1 April 1991 (01.04.91) (71) Applicant (for all designated States except US): CYTOVEN [US/US]; 1309, 114th Avenue, S.W., Suite 104, Bellevue, WA 98004 (US).	(72) Inventors; and (75) Inventors/Applicants (for US only) : KHAVINSON, Vladim mir Khatskelevich [RU/RU]; Zoologichesky Pereulok, House #1/3, Apt. #83, St. Petersburg, 197198 (RU). MOROZOV, Vyacheslav Grigorievich [RU/RU]; Zo ologichesky Pereulok, House #1/3, Apt. #83, St. Peters bourg, 197198 (RU). YAKOVLEV, German Mikhailo vich [RU/RU]; ul. Komissara Smirnova, 8-50, St. Peters burg, 194175 (RU). SEREY, Sergey Vladimirovich [RU/ RU]; 3rd Rabfak per., 12-2-11, St. Petersburg, 139012 (RU). (74) Agents: KENNEY, J., Ernest et al.; Bacon & Thomas, 625 Slaters Lane, Fourth Floor, Alexandria, VA 22314 (US). (81) Designated States: AU, CA, US. Published <i>With international search report.</i>	
(54) Title: PHARMACEUTICAL DIPEPTIDE COMPOSITIONS AND METHODS OF USE THEREOF (57) Abstract Methods are provided for the therapy of immunodeficient, immunodepressed or hyperactive immune states and for the prevention and treatment of opportunistic infections in such states comprising administering to a subject a pharmaceutically acceptable composition comprising as an active ingredient the dipeptide L-Glu-L-Trp and/or its pharmaceutically acceptable salts.		

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PHARMACEUTICAL DIPEPTIDE
COMPOSITIONS AND METHODS
OF USE THEREOF

This is a continuation-in-part of copending Serial
5 No. 07/678,129, filed April 1, 1991.

The present invention is directed to dipeptide
pharmaceutical compositions and uses thereof, in
particular, uses thereof for treatment of
immunodepressed states and of opportunistic
10 infections in immunodepressed states.

BACKGROUND OF THE INVENTION

Several polypeptides found in the thymus gland have
been implicated as playing roles in the development
and maintenance of immunological competence in
15 animals, including human beings. Some of these
polypeptides have been shown to stimulate the
maturation, differentiation and function of T-cells.
For example, a heat-stable fraction isolated from
calf thymus extracts, designated as Thymosin
20 fraction 5, has been shown to reconstitute immune
functions in thymic-deprived or immunodepressed
individuals. Several peptides have been isolated
from Thymosin fraction 5, such as Thymosin α_1 (28
amino acids, U.S. Patent No. 4,079,127), Thymosin
25 β_4 (44 amino acids, Low et al., PNAS, 78,1162-1166

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(1981)), Thymosin beta₈ (39 amino acids, U.S. Patent No. 4,389,343) and Thymosin beta₉ (41 amino acids, U.S. Patent No. 4,389,343). However, practical administration of such polypeptides is expensive due to the relatively low yield and complexity of isolation and/or manufacture of such long chain polypeptides. Most importantly, in some cases, these polypeptides produce side reactions in patients.

The present invention is based in part on the discovery that a dipeptide, hereinafter referred to as Thymogen, exhibits a broad range of efficacy for prevention and treatment of opportunistic infections in immunodepressed states, and for therapeutically effective treatment of immunodeficient states. This is believed to be highly unexpected for such a relatively small compound to exhibit such a broad range of activity. Furthermore, we have not found any significant side effects from the use of the dipeptide according to the present invention. Due to its simple nature, the dipeptide is rather inexpensive to manufacture.

As used herein, the terms "immunomodulator" and "immunomodulating" encompass the activity of enhancing or restoring the subject's immune system, as evidenced by measurable blood parameters and/or the patient's improved ability to combat infection or disease, and the ability to heal tissue. Hence, immunomodulation encompasses improvement of the immune system due to an immunodeficient state (for example, caused by removal of the thymus), and/or an immunodepressed state (for example, caused by exposure to radiation). Furthermore, the present invention provides for modulation of the immune system by lowering blood parameters and other indicia of the immune state if these indicia are abnormally

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elevated. The present invention encompasses the therapeutic method of treating the immunodeficient, immunodepressed or elevated immune state per se, thus providing prophylaxis against infection and disease,
5 as well as a treatment of infection, disease or wound indirectly by enhancing the immune system.

It is therefore an object of the present invention to provide pharmaceutical compositions of the dipeptide Thymogen which have broad immunomodulating activity,
10 as well as activity for other uses such as treatment of infections, disease and wounds (burns, frost bites, and the like), enhancement of metabolic processes, and many other uses.

It is an object of the present invention to provide
15 therapeutic methods for treatment of immunodepressed and immunodeficient states.

It is yet another object of the present invention to provide methods for preventing and treating opportunistic infections in immunodeficient and
20 immunodepressed states.

These and other objects will be apparent from the following description and appended claims.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides pharmaceutical
25 preparations comprising the dipeptide L-Glu-L-Trp, using the normal convention wherein the first named amino acid is the amino terminus and the last named amino acid is the carboxyl terminus. The compositions according to the present invention may
30 be formulated into any convenient formulation which allows for the active ingredient to be absorbed into

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the blood stream. Intramuscular and intranasal forms of application are preferred. The preferred dosage rate of the active ingredient for intramuscular administration is about 50 to 100 μ g per dose for

5 adults (for a 300 to 1000 μ g total treatment therapy); for infants up to 1 year old about 10 μ g per dose, for infants 1 to 3 years old about 10 to 20 μ g per dose; for infants 4 to 6 years old about 20 to 30 μ g per

10 dose. All of the foregoing dosages are useful for a treatment of 3 to 10 days, depending upon the immunodeficiency level. The treatment may be repeated as needed, usually within 1 to 6 months.

For prophylactic uses against opportunistic

15 infections in immunodeficient or immunodepressed patients, the intramuscular and/or intranasal single daily dose for adults may be from about 50 to 10 μ g, and for children about 10 to 50 μ g per dose for treatment over 3 to 5 days.

20 For treatment of burns, frost bite, or other wounds, including chronic apical periodontitis, the dipeptide may be applied in about 100 μ g doses as a paste or other suitable medium.

For ophthalmology, such as for treatment of

25 infectious eye diseases, the dipeptide may be applied in single daily dosages of about 10 μ g (over 4 to 10 days) or as installations into the conjunctival cavity at about 5 μ g twice daily over about 4 to 5 days.

30 The dipeptide may be utilized intramuscularly as an injection solution with the active ingredient in a therapeutically effective immunopotentiating amount of about .001 to .01% by weight. If presented in the

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form of a tablet, capsule or suppository it is preferred that the active ingredient be present in an amount of about 0.1mg per tablet, suppository or capsule. If presented in such form, the capsule, 5 suppository or tablet may also contain other conventional excipients and vehicles such as fillers, starch, glucose, etc.

The dipeptide may be obtained by conventional peptide synthesis, including the Merrifield solid state 10 peptide synthesis technique. Typically an amino and side chain protected derivative of an activated ester of glutamic acid is reacted with protected L-tryptophan. After elimination of the protecting groups and conventional purification, such as by thin 15 layer or GL chromatography, the peptide may be purified such as by, lyophilization, gel purification, and the like.

The purified dipeptide L-Glu-L-Trp, comprises a white powder (if lyophilized; otherwise, it is 20 crystalline), soluble in water, DMF; insoluble in chloroform and ether. $[\alpha]_D^{22} = +12.6$; $C = 0.5$ H₂O. $R_f = 0.65$ (butanol: acetic acid: water = 3:1:1). UV (275 \pm 5nm, max). NMR (500MHz): 0.001mol/l of the peptide solution, Trp (3.17; 3.37; 25 4.57; 7.16; 7.24; 7.71; 7.49); Glu (1.90; 1.96; 2.21; 3.72).

The active dipeptide ingredient of the pharmaceutical preparations according to the present invention may be used as a free peptide or in the form of a water 30 soluble pharmaceutically acceptable salt, such as a sodium, potassium, ammonium or zinc salt. It will be understood that the dipeptide may be administered with other active ingredients which independently